EXHIBIT D

Exponent®

Polymer Science and Materials Chemistry

Expert Report of Dr. Steven MacLean

In the U.S. District Court for the Southern District of West Virginia, Charleston Division

This document relates to:

Pelvic Mesh Litigation

In re: Ethicon Inc., Pelvic Repair System Products Liability Litigation
MDL 2327

Expert Report of
Dr. Steven MacLean

Prepared for

Chad R. Hutchinson
Butler Snow LLP
Renaissance at Colony Park
Suite 1400
1020 Highland Colony Parkway
Ridgeland, MS 39158-6010

Prepared by

Exponent
17000 Science Drive
Suite 200
Bowie, MD 20715

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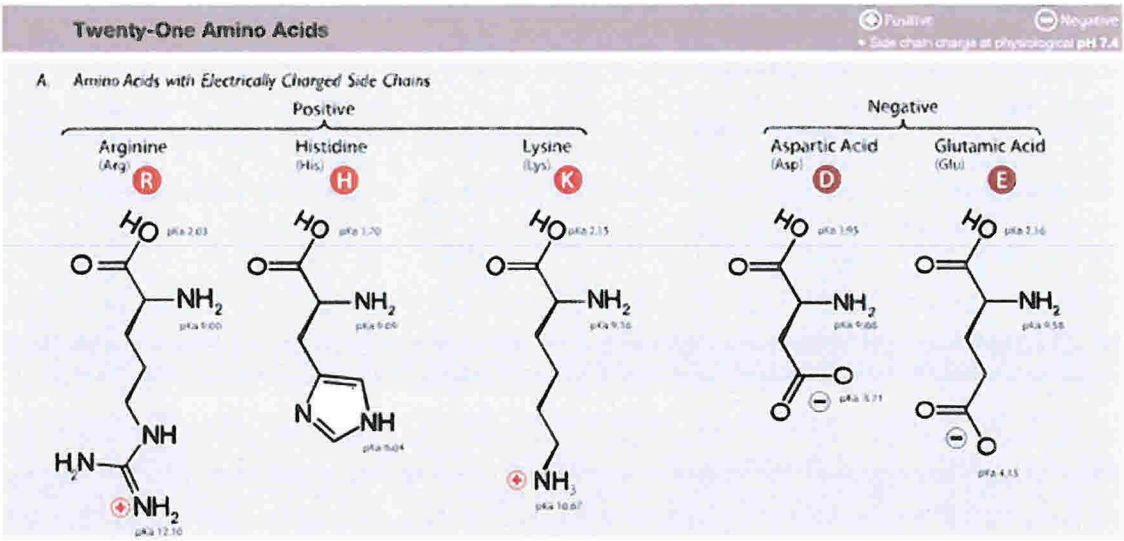

Figure 9. Amino acids that contain a net positive or negative charge.

Experimental Investigation of the Capacity of PROLENE and Oxidized PROLENE to Accept H&E Stain

In addition to my reliance on the literature and first principles of polymer science, in order to further validate my assertion that H&E is not expected to stain PROLENE or oxidized PROLENE, Exponent conducted a set of laboratory experiments that serve as the control experiments Dr. Iakovlev failed to perform in his expert report. The details of these experiments are provided below.

Sample Preparation Prior to Sectioning

Exemplar PROLENE Mesh

Pristine PROLENE mesh (Ref. No. 810041B, Lot No. 3661669) was received and kept in its original packaging until use. A clean razor blade was used to cut sections for laboratory analysis.

Chemically Oxidized PROLENE Mesh

Six sections of PROLENE mesh were oxidized according to the protocol published by Guelcher and Dunn.[63] Samples were incubated at 37°C for up to 5 weeks in oxidative media composed of 0.1 M $CoCl_2$ in 20 wt% H_2O_2. This solution purportedly simulates the oxidative environment

created by macrophages in response to a foreign object.[63] The oxidative solution was changed every 2-3 days. Prior to processing, the samples were copiously rinsed in de-ionized water, air-dried, and assessed for morphological changes using scanning electron microscopy (SEM).

QUV Oxidized PROLENE Mesh

Six sections of PROLENE mesh were placed inside a Q-Lab QUV Accelerated Weathering Tester and irradiated with 0.98 $(\frac{W}{m^2})$ UV-A and UV-B at 60°C for 5 days. As with the chemically oxidized meshes, the samples were assessed for morphological changes using SEM prior to processing.

Sample Mounting and Sectioning

Exemplar and oxidized mesh samples were embedded in both paraffin and resin (Technovit), sectioned, and stained with Hematoxylin & Eosin. All processing was performed by an independent histology lab and observed by Exponent. Detailed embedding and staining protocols can be found in Appendix A.

Paraffin-embedded samples were prepared by following the protocol submitted by Dr. Iakovlev. Briefly, samples were sequentially dehydrated in reagent alcohol and Xylene substitute using an automated tissue processor, then embedded in Leica EM400 Paraffin wax. Sections of the paraffin blocks (4-6 µm thick) were obtained using a microtome, briefly floated in a 40-45°C water bath, then mounted onto slides. Sections were air-dried for 30 minutes then baked in a 45-50°C oven overnight.

Resin-embedded samples were sequentially dehydrated in reagent alcohol using an automated tissue processor, then embedded in Technovit 7200. The polymerized resin block was trimmed, cut, and ground to a thickness of approximately 50 µm.

Paraffin and resin-embedded samples were stained with Aqueous Eosin and Harris Hematoxylin using an automated stainer. All slides were imaged by Exponent personnel using a microscope equipped with polarizing filters.

Results

SEM on Oxidized Meshes

When viewed under a Scanning Electron Microscope (SEM), the QUV-oxidized mesh exhibited external cracking (Figure 10), while the chemically-oxidized mesh did not (Figure 11). This differs from the results published by Guelcher and Dunn, who reported "pitting" and "flaking" in polypropylene meshes subjected to the same treatment conditions.[63]

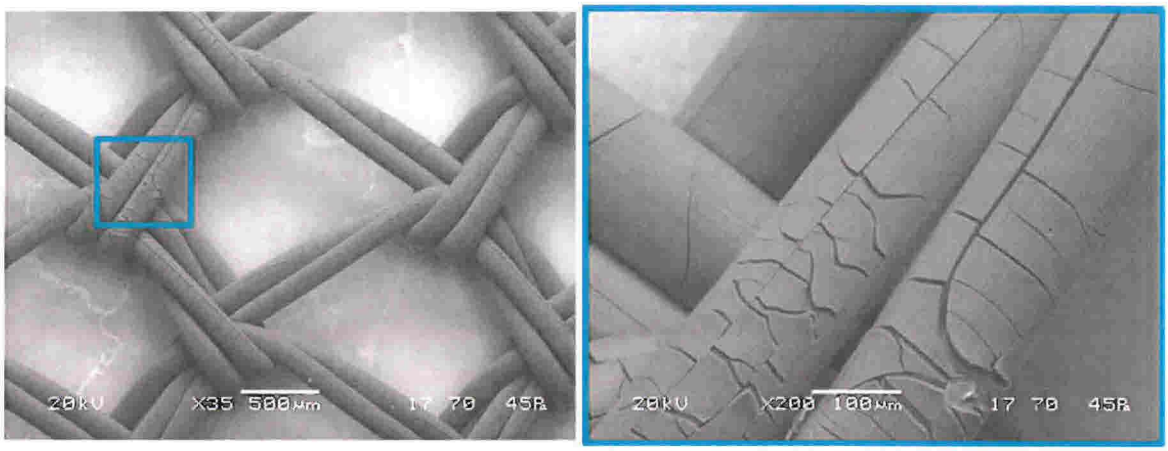

Figure 10. Scanning Electron Microscope images of QUV oxidized mesh.

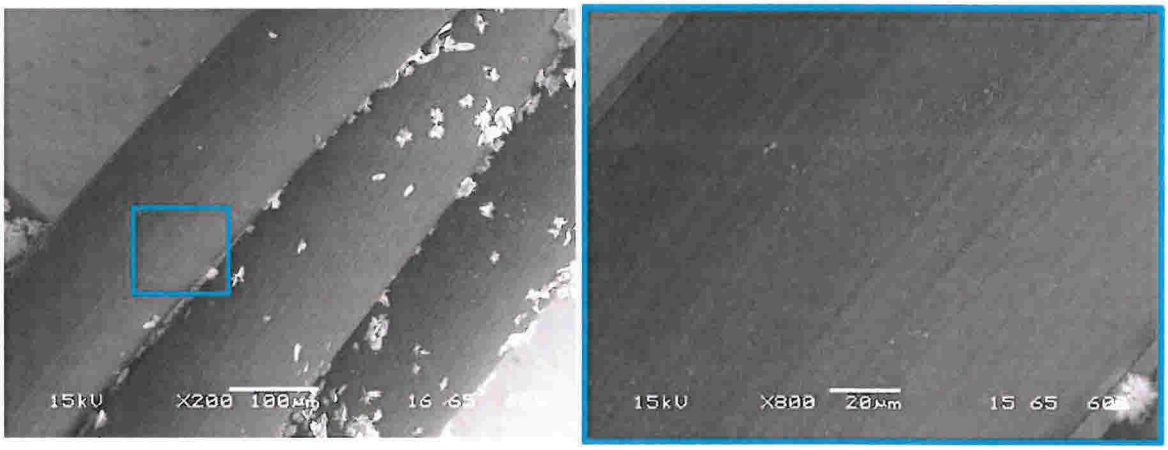

Figure 11. Scanning Electron Microscope images of mesh that was chemically-oxidized according to the Guelcher protocol.

Analysis and Experimental Validation

Six separate samples were simultaneously subjected to QUV irradiation; each contained approximately 120 individual fiber segments. While only one of these samples (Sample #2) was processed for H&E staining, it is reasonable to infer, based on SEM images of QUV-exposed samples, that a majority of the individual fibers in the treated samples were degraded, and cracked prior to being subjected to the staining process. An SEM image of Sample #4 with enumerated fiber segments used as the basis for quantifying the cracked fibers is shown below in Figure 12.

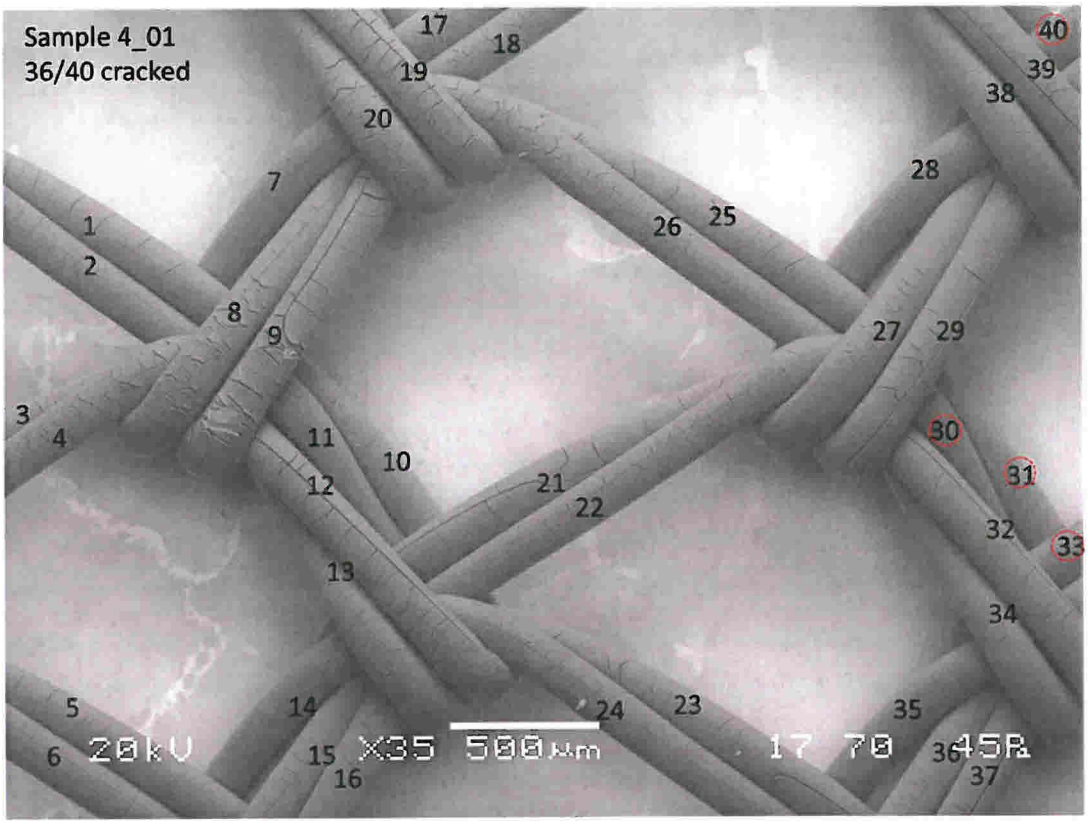

Figure 12. Scanning Electron Microscope image of QUV oxidized mesh. Numbered markings indicate individual fiber segments.

Each SEM image covers approximately one-third of the sample that was cut from the PROLENE device. The table below summarizes the findings of cracked fiber segments from portions of the three QUV-irradiated samples examined in the SEM (Samples #4, #5, and #6).

Table 3. Summary of Scanning Electron Microscope findings on QUV oxidized mesh.

Sample Number	Cracked Fiber Segments in SEM Image	Total Fiber Segments in SEM Image	% of Fiber Segments Cracked in SEM Image	95% Lower Bound on % of Cracked Fiber Segments in Sample
4	36	40	90%	80%
5	27	42	64%	52%
6	29	37	78%	66%

The rightmost column of the table presents the 95% lower confidence bound on the percentage of cracked fibers in the total sample. Although the observed percentage of cracked fiber segments varies for each of the three samples, the respective SEM images support a reliable statistical inference that, with 95% confidence, greater than half of the individual fibers in the entire sample will be cracked.

A simple conservative calculation shows that it is implausible that Sample #2 exposed to UV light and treated with histological dye contained no cracked fiber segments. Given that the treated sample contained approximately 120 fiber segments, each of which independently has a 50% probability of being cracked, the chance that none of the 120 fiber segments were cracked is only 1 in 2^{120}, or less than 1 in 10^{36} (1 followed by 36 zeroes). In other words, the probability that none of the fiber segments in Sample #2 were cracked at the time of staining is so infinitesimally small it renders the outcome, for all practical purposes, impossible.

Intentionally Oxidized PROLENE Meshes Were Not Stained by the Hematoxylin & Eosin Dyes

Positive Control – Rabbit Skin

A positive control (rabbit skin tissue) was included with the mesh samples and processed simultaneously in the automated tissue stainer, to demonstrate the effectiveness of the protocol. PROLENE meshes were subjected to the staining protocol in the same batch.

The appearance of stain is evident when tissue is present and stain has been applied. Figure 13 shows the stark contrast between rabbit tissue that has not been treated with stain (left) and

rabbit tissue that has been treated with stain (right). This experiment demonstrates that our protocol is effective in staining proteinaceous materials.

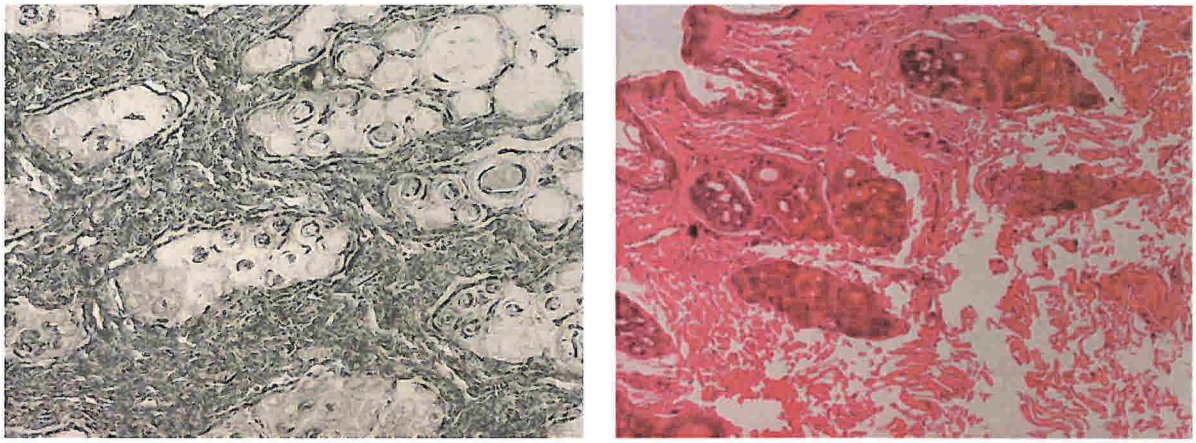

Figure 13. Processed and sectioned rabbit skin tissue not stained (left) and tissue that has been stained (right) are shown.

Non-Oxidized Control – Out-of-the-Box PROLENE Mesh

Exemplar PROLENE mesh samples with no prior exposure to laboratory UV or chemical oxidation were subjected to the Iakovlev staining protocol. As expected, the H&E stain did not bond to the PROLENE as displayed in Figure 14, confirming that the staining protocol is not effective in staining non-proteinaceous or non-ionic materials.

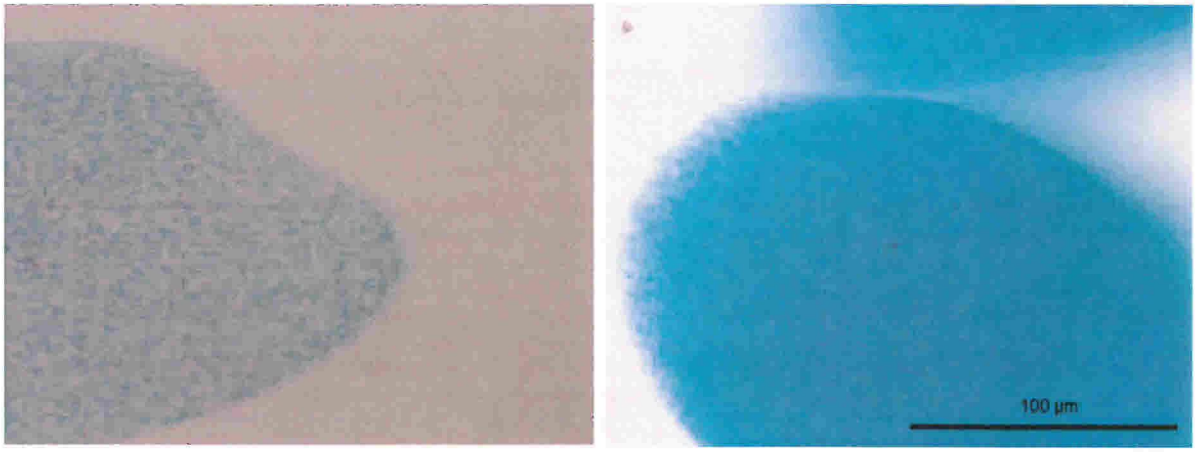

Figure 14. Pristine (exemplar) mesh embedded in paraffin (left) and resin (right), stained with H&E.

Intentionally Oxidized PROLENE – Chemical Oxidation

Exemplar PROLENE mesh samples exposed to the Guelcher chemical oxidation procedure were also subjected to the Iakovlev staining protocol. In total, twenty-two individual cross sections were examined. As shown in Figure 15, Figure 16, and Figure 17, the chemically oxidized PROLENE did not accept the H&E stain, thereby confirming the flawed methodology of Dr. Iakovlev.

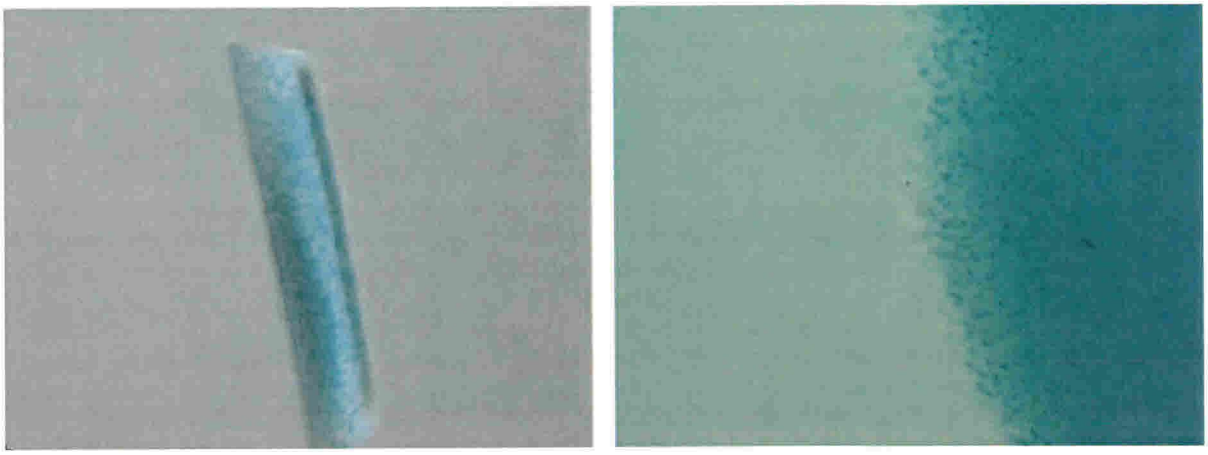

Figure 15. Chemically oxidized PROLENE mesh embedded in paraffin (left) and resin (right), stained with H&E.

An additional observation made during these experiments was that manipulation of the microscope polarizers could create a "bark-like" appearance on the fiber exterior (Figure 16 and Figure 17). This effect is likely caused by the variant thickness of the fiber across its diameter as an artifact of the sectioning process. Interestingly, what Dr. Iakovlev describes as PROLENE dye particles can be seen in the false "bark."

Figure 16. PROLENE mesh chemically oxidized with the Guelcher protocol, embedded in resin, and subjected to the H&E staining protocol. Non-polarized light (left), plane-polarized light (center), cross-polarized light (right). No staining is evident.

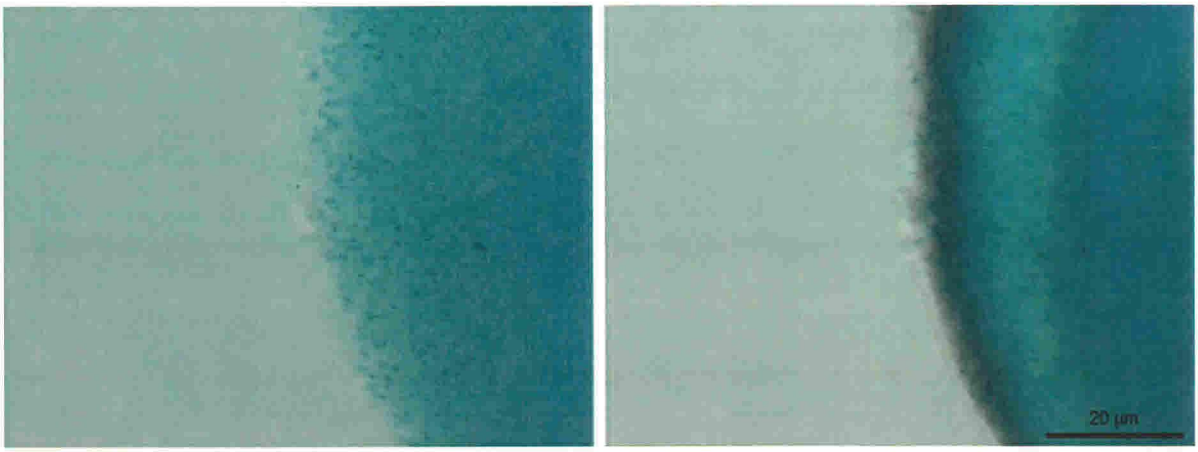

Figure 17. PROLENE mesh chemically oxidized with the Guelcher protocol, embedded in resin, and subjected to the H&E staining protocol. Non-polarized light (left), plane-polarized light (right). No staining is evident.

Intentionally Oxidized PROLENE – UV Oxidation

Exemplar PROLENE mesh samples exposed to QUV oxidation were also subjected to the Iakovlev staining protocol. In total, over one hundred individual cross sections were examined. As shown in Figure 18, the chemically oxidized PROLENE did not accept the H&E stain. In addition, as shown in Figure 19, despite the fact that the fiber was cracked, and according to Dr. Iakovlev should have physically trapped stain, the QUV oxidized PROLENE did not accept the H&E stain, thereby again confirming Dr. Iakovlev's flawed methodology. Despite multiple observations using high and low magnifications, polarized and non-polarized light, no evidence of the stain being trapped, captured, or otherwise bound within the cracks of the damaged mesh was observed.

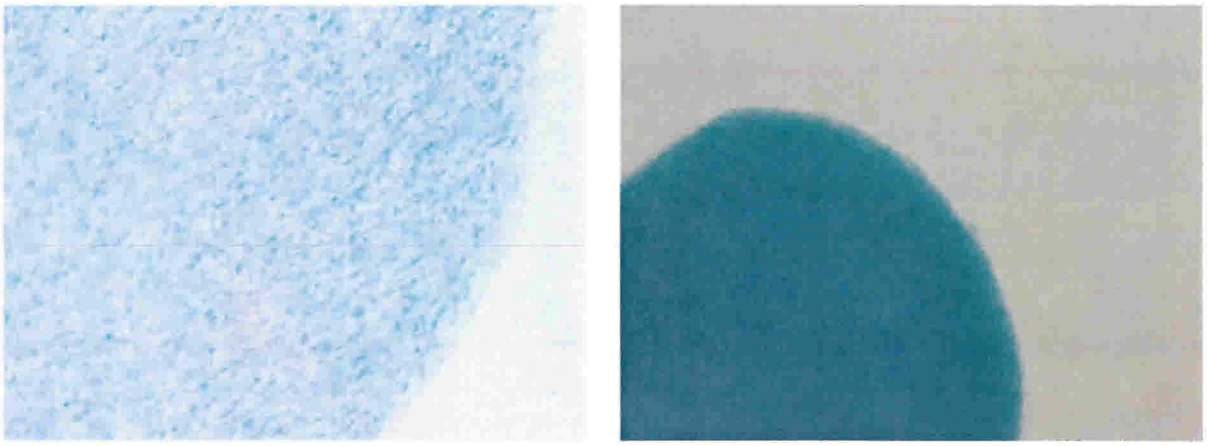

Figure 18. QUV oxidized PROLENE mesh embedded in paraffin (left) and resin (right), stained with H&E.

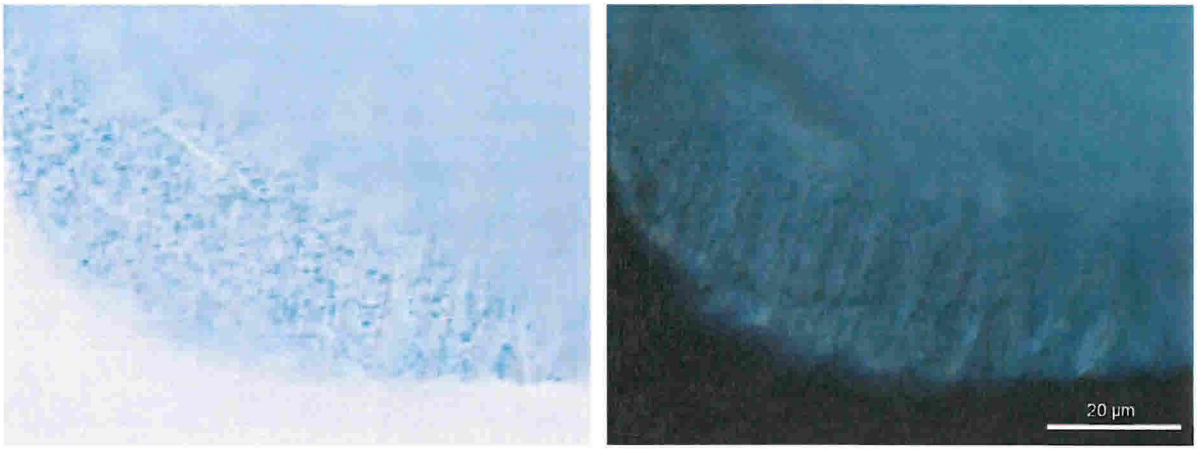

Figure 19. QUV treated mesh exhibiting several cracks but no evidence of H&E stain. Image on the left was acquired in absence of polarization, the image on the right was taken with polarization.

Statistical Significance

A statistical analysis of my control experiment was scientifically unnecessary. In the microscopy experiments conducted, intentionally oxidized PROLENE was shown to not hold stain. No variability was observed in these results, meaning none of the nearly 150 individual fiber segment cross sections prepared and examined according to Dr. Iakovlev's staining protocol were found to have stained. The lack of variability in the experimental data supports the conclusion that these observations are essentially deterministic in nature, reproducible

exactly or with negligible variability. As defined in *A Dictionary of Computing*, published by the Oxford University Press,[106] statistical methods are:

> "Methods of collecting, summarizing, analyzing, and interpreting variable numerical data. Statistical methods can be contrasted with deterministic methods, which are appropriate where observations are exactly reproducible or are assumed to be so."

In view of this definition, which distinguishes statistical from deterministic methods, the omission of a formal analysis—i.e., an application of statistical methods to the unvarying data in this experiment—is appropriate and consistent with generally accepted scientific practice.

Imaging Artifacts

Microtome slicing of polymeric samples is a technique with which I am familiar, and has been used in the field of polymer science for decades.[107,108,109] Observation of thin-sliced polymeric specimens, including those that have been dyed, requires an understanding of potential artifacts that can exist as a result of the cutting and imaging process.

Thickness Variation and Stain Pooling

When high aspect ratio samples (such as fibers) are sectioned with a microtome, simple geometry dictates that the thickness will be variant if the microtome knife is not completely orthogonal to the sample's long axis. This geometric artifact is exhibited schematically in Figure 20A-D, which illustrates that the edges of the sliced specimen are thinner when viewed under the microscope.

This same effect can result in stain pooling, which is also illustrated schematically in Figure 20. The cylindrical fibers that compose the mesh (A) can be cut in an oval shape depending on the

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[106] *A Dictionary of Computing,* Oxford University Press, 2004.

[107] Wang, X., Zhou, W. Glass Transition of Microtome-Sliced Thin Films. *Macromolecules*, (2002) 35(18):6747–6750.

[108] Stiftinger, M., Buchberger, W., Klampfl, C. W. Miniaturised Method for the Quantitation of Stabilisers in Microtome Cuts of Polymer Materials by HPLC with UV, MS or MS2 Detection. *Anal. Bioanal. Chem.*, (2013) 405(10):3177–3184.

[109] Janeschitz-Kriegl, H., Krobath, G., Roth, W., Schausberger, A. On the Kinetics of Polymer Crystallization under Shear. *Eur. Polym. J.*, (1983) 19(10-11):893–898.

angle at which the blade encounters the block (B). When the resulting section (C) is placed upon a glass slide and stained, the angle between the section and the glass forms a small pocket in which stains can accumulate (D), giving the appearance, including color, of "true" staining (E) – that is, of chemical interactions between dyes and their ligands. In reality, this is merely a mechanical entrapment of the staining solution creating an artifact and does not indicate staining of the sample being examined.

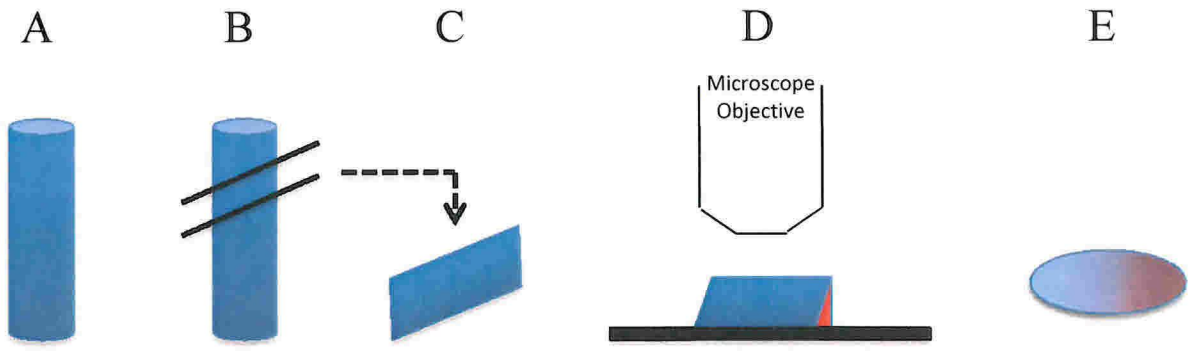

Figure 20. Potential formation mechanism of pooling artifact. A mesh fiber (A) can encounter the microtome blade at an angle (B), forming a section with an angled ledge (C), under which stain can pool (D) and give the appearance of true staining (E).

Polarizing Artifact

Polarized microscopy is a powerful tool in polymer science.[110] With good optics and proper alignment, it allows for the visualization of anisotropic structures, making them appear under varying shades of brightness with a polarizing filter in the microscope's light path.[111] The brightness of the sample when imaged under polarization depends on factors such as sample alignment. The brightness is highest when the object is aligned at a 45° angle to the polarizers. On the other hand, the object can become difficult to see when aligned parallel to one of the two polarization planes.[111]

[110] Lenz, R. W. Experiments in Polymer Science, Edward A. Collins, Jan Bares, Fred W. Billmeyer, Jr., Wiley-Interscience, New York, 1973. 530 Pp. $16.95. *J. Polym. Sci. Polym. Lett. Ed.*, (1974) 12(9):535–536.

[111] Wolman, M. Polarized Light Microscopy as a Tool of Diagnostic Pathology. *J. Histochem. Cytochem.*, (1975) 23(1):21–50.

The aforementioned thickness variation resultant from microtoming, as well as the tendency of an anisotropic fiber to tear away from a surrounding matrix, can create edge artifacts under polarized light. An example of such an artifact is displayed in Figure 21 (as well as Figure 16 and Figure 17), which is a micrograph of a non-oxidized (no possible "bark") exemplar PROLENE mesh fiber subjected to the H&E staining protocol. In Figure 21A, the fiber is shown under polarized light, and a dark ring of false "bark" is visible on a portion of the fiber exterior. Figure 21B and C are the same sample area at higher magnification, with and without the polarizer, respectively.

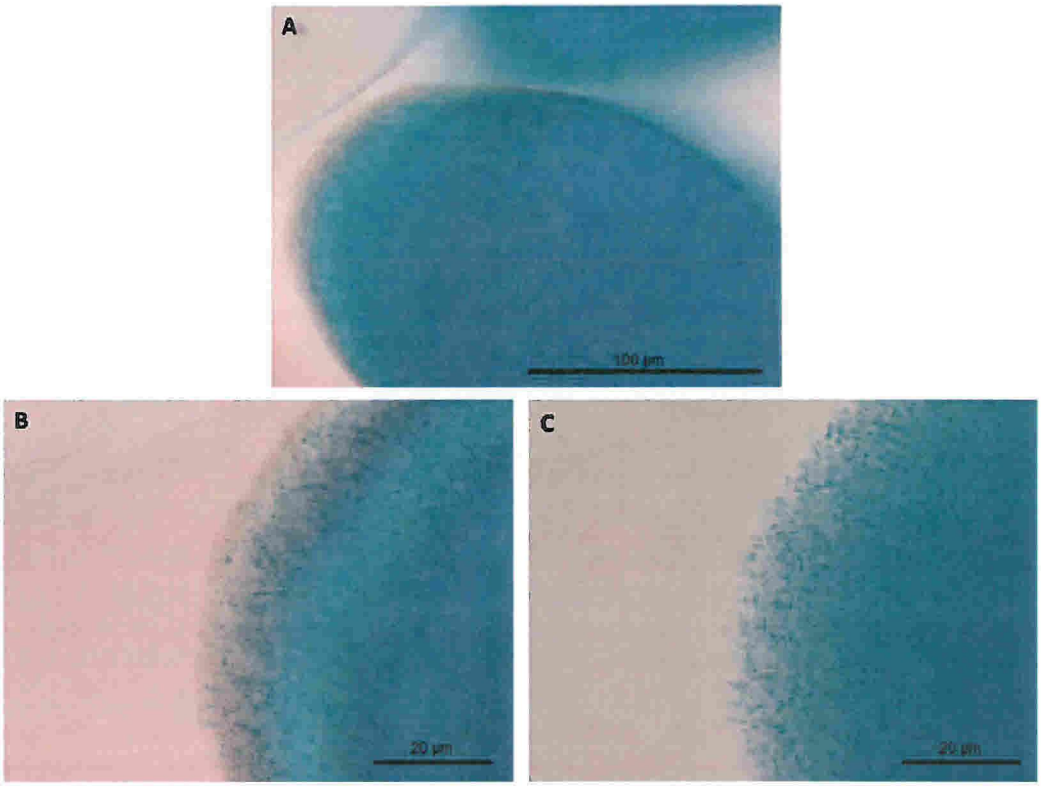

Figure 21. Exemplar, unoxidized, PROLENE mesh after staining with H&E. Images A and B were acquired with plane-polarized light, image C was obtained without polarized light.

Becke Lines

Differences in refractive indices (material density) between a transparent specimen and its surroundings can cause the appearance of light and dark parallel lines at the interface between

materials. These lines are known in microscopy as Becke lines.[112] Figure 22 illustrates this imaging artifact at the edge of a QUV oxidized PROLENE mesh cross section embedded in paraffin and subjected to H&E staining. The relative location and thickness of these lines change based on the position of the focal plane with respect to the surface of the microscopy slide, with the thinnest line corresponding to the microscope being focused at the surface. These lines can be altered by changing the focus in the microscope[112] as seen in the videos included in Appendix F.

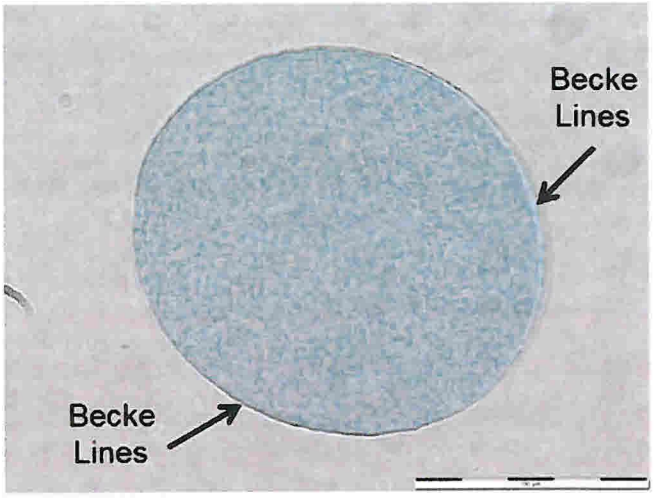

Figure 22. QUV oxidized PROLENE mesh embedded in paraffin, and subjected to the H&E staining protocol. Notice dark and light Becke lines around the fiber parameter.

Purple Hue

Additional imaging artifacts can be created due to changes in incident light. For example, the artificial faint purple hue observed in discrete locations of the specimen shown in Figure 23 (right) is the consequence of exposing the specimen to plane-polarized light. It is evident that this hue is an artifact of the imaging technique due to its absence when the same specimen is examined with the same microscope at the same magnficiation under non-polarized light (left).

[112] Zalevsky, Z., Sarafis, V., "Phase Imaging in Plant Cells and Tissues," *Biomedical Optical Phase Microscopy and Nanoscopy,* chapter 4. Oxford, UK: Elsevier, 2013.

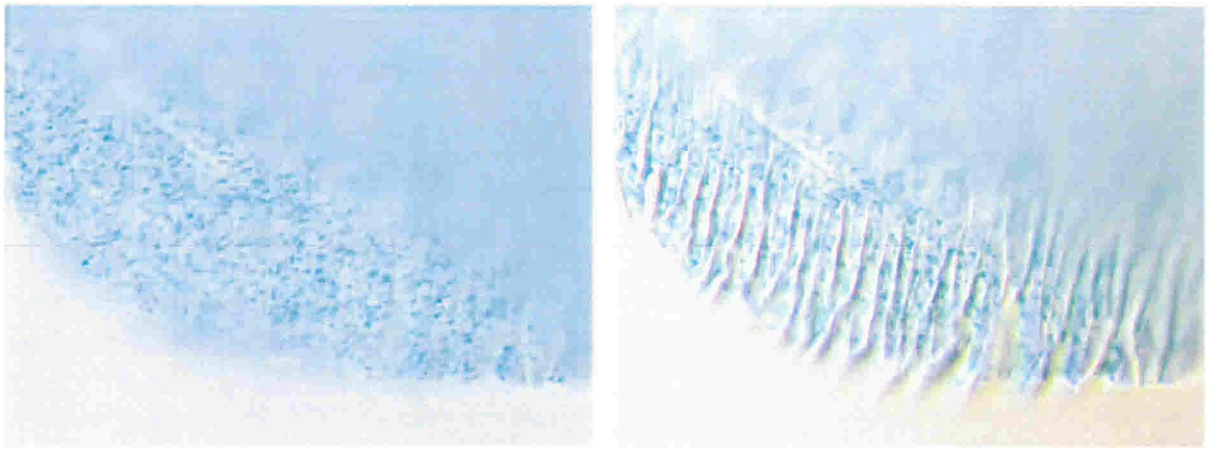

Figure 23. QUV oxidized PROLENE mesh embedded in paraffin, subjected to the H&E staining protocol, and imaged with non-polarized light (left) and plane-polarized light (right). No staining is evident. The purple hue (right) is an artifact of the imaging technique.

Blue Granules Outside Fiber Boundary

Due to the cylindrical nature of the fibers, if a cross section is not cut perfectly orthogonal to the fiber's long axis, the resulting slice will be slightly skewed as seen in Figure 24 (left). When this fiber is viewed from the top, through the microscope the blue granules beneath the top surface of the fiber cross section appear to extend beyond the fiber boundary, as shown schematically in Figure 24 (right). The Becke lines dicate the outline of the fiber at the surface of the microscope slide. An example of this artifact can be seen in Figure 25, where an unoxidized exemplar PROLENE mesh was mounted in paraffin, cross sectioned and subjected to the H&E staining protocol. When examined with the optical microscope, the fiber exhibits this artifact (i.e. has the appearance of blue granules extending beyond the fiber boundaries).

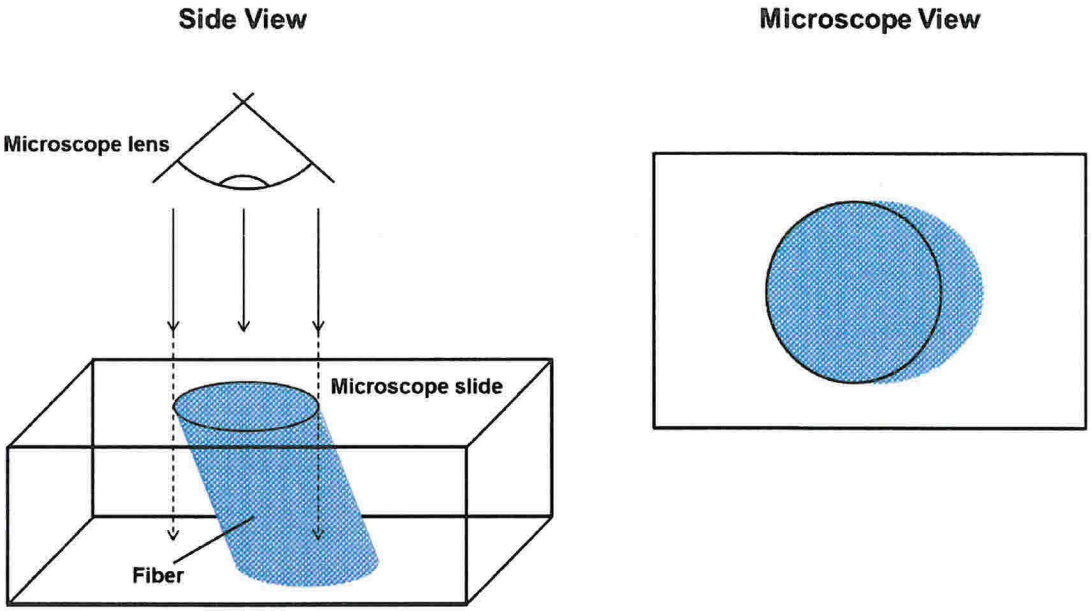

Figure 24. Schematic of potential artifact mechanism where blue granules appear outside the fiber boundary. If a fiber is cut at an angle (left) and the cross section is viewed from the top through the microscope lens (right), it can appear that the blue granules extend beyond the fiber boundary.

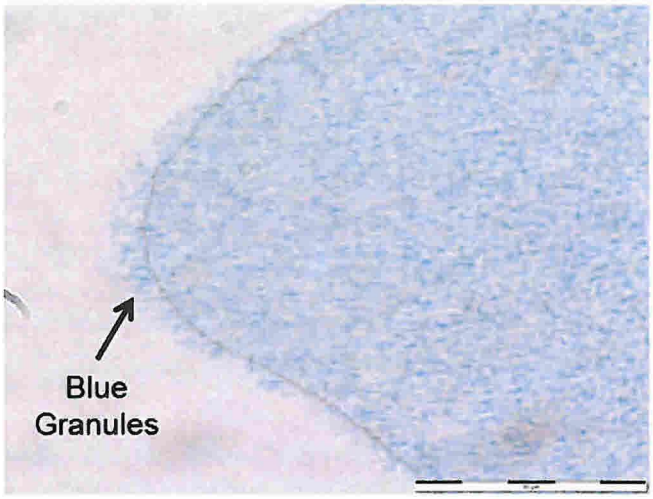

Figure 25. Unoxidized exemplar PROLENE mesh embedded in paraffin, and subjected to the H&E staining protocol. Note the appearance of blue granules beyond the exterior of the fiber.

- Dr. Iakovlev has not performed any control experiments nor cited any scientific studies that support his belief that degraded PROLENE is capable of being histologically stained with H&E stains, and for these reasons, his conclusions are flawed and suspect.

- Through a series of controlled oxidation, microtoming, and microscopy experiments, Exponent demonstrated that oxidized PROLENE meshes do not become stained with H&E dyes. This fact is supported by polymer science first principles, given that PROLENE does not possess chemical groups amenable to binding with the H&E stain molecules.

- Artifacts can be easily introduced during sample preparation, sectioning, staining, and imaging, giving the appearance of darkened outer layers.

- A brittle outer layer will not contribute to the stiffness of the mesh if it is thin, cracked, and discontinuous. Dr. Iakovlev's opinion that a thin, cracked, porous outer layer causes an increase in mesh stiffness is not consistent with first principles of polymer science and solid mechanics.

If you have any questions or require additional information, please do not hesitate to contact me.

Steven MacLean, Ph.D., P.E.
Senior Managing Engineer